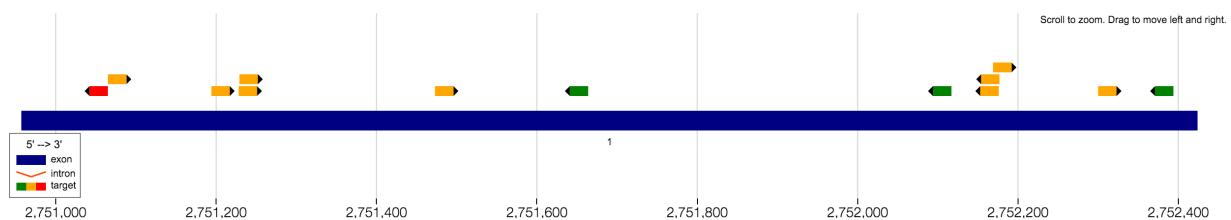
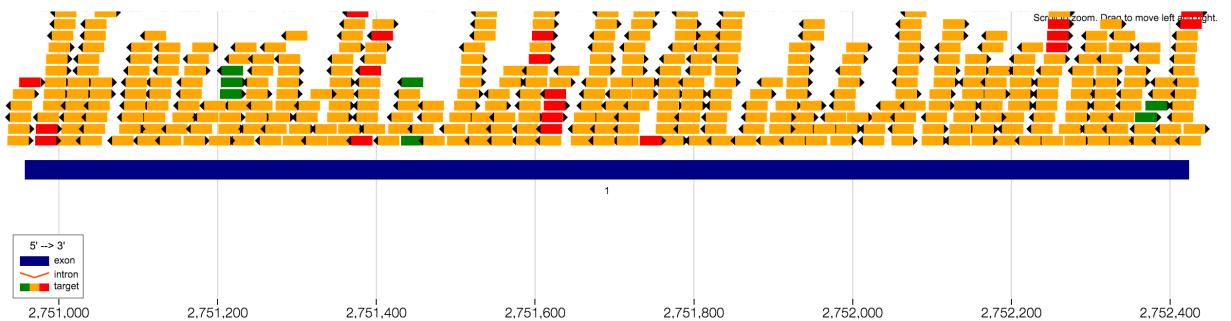


Supplementary Figure 1: Increased range of targeting in CHOPCHOP v2. Targeting the zebrafish gene *Apela* with the SpCas9 CRISPR effector (NGG PAM, Xu et al. scoring) yields no good sgRNAs (upper panel, only red). Targeting with a modified Cas9 (NGA PAM, lower panel) yields two good sgRNAs (green), and 4 adequate sgRNAs (yellow).

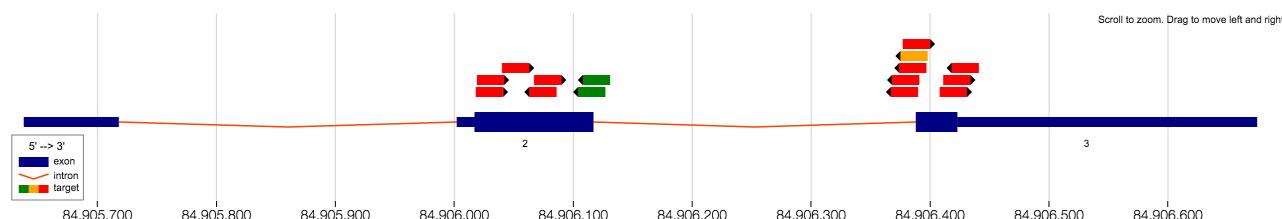
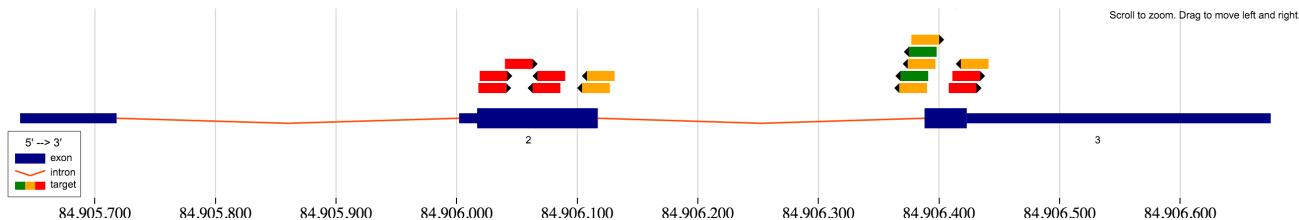
PF3D7_1467300



PF3D7_1467300



Supplementary Figure 2: Targeting genes in an AT-rich genome with Cpf1. The GC-content of *P. falciparum* is only 23%. The standard Cas9 (upper panel) requires a G-rich PAM site (NGG) which may severely impede the search for sgRNAs in such genomes. In these cases alternative CRISPR effectors like Cpf1 that have a T-rich PAM (TTN) enables efficient targeting (lower panel).



Supplementary Figure 3: Comparison of scoring in CHOPCHOP v1 versus v2. Example human gene *TMSB10* targeted by CHOPCHOP v1 (upper panel) and v2 (lower panel, Xu scoring scheme). Due to a large number of 3 mismatch off-targets for the top 2 sgRNAs in v1, those sgRNAs are now demoted in v2. Conversely, in v2, two sgRNAs lacking a G at position 20 are promoted due to having reasonable efficiency under the Xu model and no predicted off-targets. Scoring for individual sgRNAs and a comparison of the sgRNAs is found in Supplementary Table 2.

CRISPR effector	PAM	Publication
<i>Streptococcus pyogenes</i>	NGG-3'	Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J.A., Charpentier, E. (2012) A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. <i>Science</i> 337(6096): 816–821.
<i>Streptococcus pyogenes</i>	NAG-3'	Kleinberger,B.P., Pattanayak,V., Prew,M.S., Tsai,S.Q., Nguyen,N.T., Zheng,Z. and Keith Joung,J. (2016) High-fidelity CRISPR-Cas9 nucleases with no detectable genome-wide off-target effects. <i>Nature</i> 529, 490–495
<i>Streptococcus pyogenes</i>	NGA-3'	Kleinberger,B.P., Pattanayak,V., Prew,M.S., Tsai,S.Q., Nguyen,N.T., Zheng,Z. and Keith Joung,J. (2016) High-fidelity CRISPR-Cas9 nucleases with no detectable genome-wide off-target effects. <i>Nature</i> 529, 490–495
<i>Streptococcus thermophilus</i>	NNAGAAW-3'	Sapranauskas, R., Gasiunas, G., Fremaux, C., Barrangou, R., Horvath, P., & Siksnys, V. (2011). The <i>Streptococcus thermophilus</i> CRISPR/Cas system provides immunity in <i>Escherichia coli</i> . <i>Nucleic Acids Research</i> , 39(21), 9275–9282
<i>Neisseria meningitidis</i>	NNNNNGMTT-3'	Hou, Z., Zhang, Y., Propson, N. E., Howden, S. E., Chu, L.-F., Sontheimer, E. J., & Thomson, J. A. (2013). Efficient genome engineering in human pluripotent stem cells using Cas9 from <i>Neisseria meningitidis</i> . <i>Proceedings of the National Academy of Sciences of the United States of America</i> , 110(39), 15644–15649
<i>Staphylococcus aureus</i>	NNGRRT-3'	Ran, F. A., Cong, L., Yan, W. X., Scott, D. A., Gootenberg, J. S., Kriz, A. J., ... Zhang, F. (2015). In vivo genome editing using <i>Staphylococcus aureus</i> Cas9. <i>Nature</i> , 520(7546), 186–191
<i>Acidaminococcus, Lachnospiraceae</i>	5'-TTTN	Zetsche,B., Gootenberg,J.S., Abudayyeh,O.O., Slaymaker,I.M., Makarova,K.S., Essletzbichler,P., Volz,S.E., Joung,J., van der Oost,J., Regev,A., et al. (2015) Cpf1 Is a Single RNA-Guided Endonuclease of a Class 2 CRISPR-Cas System. <i>Cell</i> 163.3 (2015): 759-771.
<i>Prevotella, Francisella</i>	5'-TTN	Zetsche,B., Gootenberg,J.S., Abudayyeh,O.O., Slaymaker,I.M., Makarova,K.S., Essletzbichler,P., Volz,S.E., Joung,J., van der Oost,J., Regev,A., et al. (2015) Cpf1 Is a Single RNA-Guided Endonuclease of a Class 2 CRISPR-Cas System. <i>Cell</i> 163.3 (2015): 759-771.

Supplementary Table 1: Overview of the CRISPR effectors and the PAMs supported by CHOPCHOP. In addition, CHOPCHOP supports custom PAMs at either the 5' or 3' end which can be specified with the IUPAC alphabet.

Ranking	Target sequence	Genomic location	Exon	Strand	GC (%)	Off-targets		
						0	1	2
1	TGGCTGTGGAGAGCGGGAAAGCGG	chr2:84906369	3	"_"	70	0	0	0
2	TGCTCAATGGCTGTGGAGAGCGG	chr2:84906376	3	"_"	61	0	0	0
3	GCACACTCACTCTTTGGTCGG	chr2:84906105	2	"_"	57	0	0	0
4	CGAGGCACACTCACTCTTGG	chr2:84906109	2	"_"	57	0	0	0
5	GCTCTCCACAGCATTGAGCAGG	chr2:84906378	3	"+"	61	0	0	0
6	GGCTGTGGAGAGCGGGAAAGCGG	chr2:84906368	3	"_"	74	0	0	2
7	GCTCAATGGCTGTGGAGAGCGG	chr2:84906375	3	"_"	65	0	0	2
8	AGGAAATCCTCAGGATCTTAGG	chr2:84906419	3	"_"	48	0	0	2
9	GAAATTCTCTAAGATCCTGGAGG	chr2:84906412	3	"+"	43	0	1	1
10	GGAAATCCCAGCTCGATAAGG	chr2:84906041	2	"+"	52	0	1	0
11	TGGCAGACAAACCAGACATGGGG	chr2:84906019	2	"+"	57	0	3	0
12	GGCAGACAAACCAGACATGGGG	chr2:84906020	2	"+"	61	0	3	0
13	AGTGAATTCTCTAAGATCCTGG	chr2:84906409	3	"+"	39	0	1	2
14	CGTCTCGTTTCTCAGCTTGG	chr2:84906064	2	"_"	52	1	0	1
15	GCTGAAGAACCGGAGACGCAGG	chr2:84906068	2	"_"	57	1	0	1

Ranking	Target sequence	Genomic location	Exon	Strand	GC (%)	Self-complementarity	Off-targets			Efficiency
							0	1	2	
1	GCACACTCACTCTTTGGTCGG	chr2:84906105	2	"_"	57	1	0	0	0	0.41
2	CGAGGCACACTCACTCTTGG	chr2:84906109	2	"_"	57	0	0	0	0	0.31
3	TGCTCAATGGCTGTGGAGAGCGG	chr2:84906376	3	"_"	61	0	0	0	2	0.58
4	GCTCTCCACAGCATTGAGCAGG	chr2:84906378	3	"+"	61	1	0	0	5	0.59
5	GGAAATCGCCAGCTCGATAAGG	chr2:84906041	2	"+"	52	1	0	1	0	0.53
6	CGTCTCGTTTCTCAGCTTGG	chr2:84906064	2	"_"	52	0	1	0	1	0.4
7	TGGCAGACAAACCAGACATGGGG	chr2:8490619	2	"_+"	57	0	0	3	0	0.62
8	AGGAAATCCTCAGGATCTTAGG	chr2:84906419	3	"_+"	48	2	0	0	2	0.29
9	GCTGAAGAACCGGAGACGCAGG	chr2:84906068	2	"+"	57	2	1	0	1	0.67
10	GGCAGACAAACCAGACATGGGG	chr2:84906020	2	"+"	61	0	0	3	0	0.61
11	AGTGAATTCTCTAAGATCCTGG	chr2:84906409	3	"+"	39	0	0	0	2	0.47
12	GAAATTCTCTAAGATCCTGGAGG	chr2:84906412	3	"+"	43	1	0	1	1	0.59
13	GCTCAATGGCTGTGGAGAGCGG	chr2:84906375	3	"_+"	65	0	0	0	2	0.48
14	TGGCTGTGGAGAGCGGGAAAGCGG	chr2:84906369	3	"_+"	70	0	0	0	0	0.67
15	GGCTGTGGAGAGCGGGAAAGCGG	chr2:84906368	3	"_+"	74	0	0	0	2	0.54

Ranking in version 1	Ranking in version 2	Cause of difference
1	14	Although it has a high efficiency score, CHOPCHOP v2 found 13 off-targets with 3 mismatches for this sgRNA
2	3	This sgRNA has a reasonable efficiency score, but CHOPCHOP v2 found 2 off-targets with 3 mismatches
3	1	These sgRNAs have a modest efficiency score, but they have no predicted off-targets so are considered the best. Please note: sgRNA #1 (in v2) has one 4 nt region of self-complementarity, but since this is a novel scoring metric, it currently carries only a small penalty.
4	2	CHOPCHOP v2 is more restrictive about off-targets and considers 5 off-targets with 3 mismatches to be a poor sgRNA
5	4	CHOPCHOP v2 demoted these sgRNAs due to the large number of 3 mismatch off-targets
6	15	CHOPCHOP v2 identified additional 3 mismatch off-targets for this sgRNA, as well as incidents of self-complementarity
7	13	While some of these sgRNAs have reasonable efficiency scores, all of them have too many off-targets to be considered adequate
8	8	
9	12	
10	5	
11	7	
12	10	
13	11	
14	6	
15	9	

Supplementary Table 2: Results from CHOPCHOP v1 (upper table) and v2 using the Xu et al. model for scoring (middle table) on the human gene TMSB10 displayed in Supplemental Figure 3. A comparison and explanation for the differences in ranking between the two versions can be found in the lower table.